Safety and immunogenicity of co-administered hookworm vaccine candidates *Na*-GST-1 and *Na*-APR-1 in Gabonese adults: a randomised, controlled, double-blind, phase 1 dose-escalation trial

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Summary

Background Hookworms cause substantial morbidity in children and women of reproductive age. The control strategy of mass drug administration is suboptimal, hence the need for a vaccine. *Necator americanus* aspartic protease-1 (*Na*-APR-1) and *N americanus* glutathione S-transferase-1 (*Na*-GST-1) are involved in the digestion and detoxification of haemoglobin in the hookworm digestive tract. In animal models, vaccination against these antigens resulted in protection from challenge infection. Both vaccine candidates were shown to be safe and well tolerated when administered separately to healthy adults. We assessed the safety and immunogenicity of co-administered *Na*-GST-1 and *Na*-APR-1 (M74) vaccines in healthy Gabonese adults.

Methods This randomised, controlled, double-blind, phase 1, dose-escalation trial was done at the Centre de Recherches Médicales de Lambaréné, in a region of Gabon where *N americanus* and other helminths are prevalent. Healthy adults aged 18–50 years and living in Lambaréné or the surrounding areas were recruited to the study. Participants were enrolled consecutively into two dose cohorts (30 µg or 100 µg of the experimental vaccines) and randomly assigned in blocks (block size four) to receive three doses of either co-administered *Na*-GST-1 plus *Na*-APR-1 (M74; 30 µg or 100 µg of each), adjuvanted with Alhydrogel (aluminium hydroxide gel suspension) together with an aqueous formulation of glucopyranosyl lipid A, or hepatitis B vaccine plus saline (control group). Vaccines were administered intramuscularly on days 0, 28, and 180. The primary endpoint was safety, with immunogenicity a secondary endpoint. The intention-to-treat population was used for safety analyses, whereas for immunogenicity analyses, the per-protocol population was used (participants who received all scheduled vaccinations). Control vaccine recipients for both dose cohorts were combined for the analyses. The trial is registered with ClinicalTrials.gov, NCT02126462.

Findings Between Oct 27, 2014, and Jan 31, 2015, 56 individuals were screened for eligibility, of whom 32 were enrolled and randomly assigned to one of the three study groups (12 each in the 30 µg and 100 µg experimental vaccine groups and eight in the control group). Both study vaccines were well tolerated in both dose groups. The most common adverse events were mild-to-moderate injection-site pain, headache, myalgia, and nausea. No severe or serious adverse events related to the vaccines were recorded. 52 unsolicited vaccine-related adverse events occurred during the study, but there was no difference in frequency between vaccine groups. IgG antibodies were induced to each of the vaccine antigens, with mean IgG levels increasing after each vaccination. Vaccination with 100 µg of each vaccine antigen consistently induced IgG seroconversion (IgG levels above the reactivity threshold). Peak IgG responses were observed 2 weeks after the third vaccine dose for both antigens, with all participants who received the 100 µg doses seroconverting at that timepoint. IgG levels steadily declined until the final study visit 6 months after the third vaccination, although they remained significantly higher than baseline in the 100 µg dose group.

Interpretation Vaccination with recombinant *Na*-GST-1 and *Na*-APR-1 (M74) in healthy adults living in *N americanus*-endemic areas of Gabon was safe and induced IgG to each antigen. To our knowledge, this study is the first to report results of *Na*-APR-1 (M74) co-administered with Alhydrogel in participants from an *N americanus*-endemic area. Further clinical development of these vaccines should involve efficacy studies.

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Research in context

Evidence before this study

Around 450 million people are infected with hookworms, mostly in low-income countries in tropical and subtropical parts of the world. There are no licensed vaccines to prevent hookworm. *Na*-GST-1 and *Na*-APR-1 (M74) are the current lead vaccine candidates. Vaccination with these antigens is hypothesised to induce neutralising antibodies that will interfere with the functions of the native proteins, inducing parasite death or impairing worm fecundity, thereby reducing or interrupting transmission. Both vaccines have been found to be well tolerated and safe in clinical trials in which the antigens were administered separately to healthy adults. Based on preclinical studies, it is expected that more than a single necator antigen will be required for an effective vaccine. Therefore, clinical development of the human hookworm vaccine has assumed incorporation of at least two antigens into a final, coformulated product. Co-administration of *Na*-GST-1 and *Na*-APR-1 (M74) is meant to guide the choice of dose of each antigen for production of coformulated product. We searched PubMed and the Cochrane Library for research articles published between Jan 1, 1980, and March 5, 2020, using the terms "hookworm", "vaccine", "clinical trial", and "phase". No language restrictions were applied. The four manuscripts identified included three that reported the results of phase 1 trials with long-term safety or immunogenicity follow-up; only one study tested either *Na*-GST-1 or *Na*-APR-1 (M74). Vaccinations in this trial of *Na*-GST-1 were safe, well tolerated, and resulted in significant antigen-specific IgG responses.

Added value of this study

To our knowledge, our study describes the first phase 1 clinical trial of two novel vaccine candidates against human hookworm infection (*Na*-APR-1 [(M74]) and *Na*-GST-1) done in sub-Saharan Africa. It also describes, for the first time, the safety and immunogenicity of *Na*-APR-1 (M74) administered to healthy adult residents of an *N americanus*-endemic area.

Implications of all the available evidence

A vaccine against hookworm could be used to prevent disease in the global population, with high-risk populations such as children and women of childbearing potential prioritised to receive vaccination. Immune correlates of protection against hookworm disease have not yet been determined. Co-administration of *Na*-GST-1 and *Na*-APR-1 (M74) resulted in rapid induction of humoral immune responses against both vaccine antigens, with highest responses detected 2 weeks after the third dose of each antigen, although antibody response declined over the following 8 months. Altogether, the safety and immunogenicity evidence justifies progression to phase 2 and 3 clinical trials, including studies in younger age groups.

Introduction

Around 450 million people are infected with hookworms, largely in low-income and middle-income countries in tropical and subtropical parts of the world,[1] leading to considerable economic and health burdens.[2] Hookworms penetrate the skin after contact with soil contaminated with human faeces. Once inside the human body, larvae migrate to the gastrointestinal tract, where they become adult worms that attach to the mucosa of the small intestine and feed on blood, causing a loss of host blood and iron.[3] Chronic, heavy infections cause so-called hookworm disease, consisting mainly of iron-deficiency anaemia.[4] Because of their lower iron stores, women of reproductive age and children are particularly vulnerable to iron-deficiency anaemia, which causes impaired intellectual and physical development, adverse birth outcomes, and reduced economic productivity.[4] Through these mechanisms, hookworm infection results in an estimated 4·1 million disability-adjusted life-years and global economic losses of up to US$140 billion annually.[2]

The primary hookworm control measure is periodic mass drug administration using benzimidazole anthelmintics.[3] However, this strategy yields only suboptimal cure rates,[5] and reinfection occurs rapidly. Moreover, mass drug administration does not significantly reduce the prevalence of hookworm infection,[6] nor of hookworm-related anaemia.[7] Prevention of infections of moderate and heavy intensity with a vaccine could overcome the limitations of mass drug administration[8,9] and could provide substantial, cost-effective health benefits in high-transmission settings.[10]

Hookworms survive by ingesting host blood, lysing erythrocytes, and digesting haemoglobin via a proteolytic cascade in their digestive tracts.[11,12] *Necator americanus* aspartic protease-1 (*Na*-APR-1) initiates this cascade in *N americanus* hookworms,[11] whereas glutathione S-transferase-1 in the same species (*Na*-GST-1) binds and detoxifies oxygen radicals generated by free haem released during haemoglobin digestion.[13–15] We hypothesise that vaccination with these antigens, produced as recombinant proteins, will induce neutralising antibodies that will interfere with the functions of the native proteins, inducing parasite death or impairing worm fecundity, thereby reducing or interrupting transmission.[16]

Hookworm haemoglobinase recombinant subunit vaccines have resulted in significantly lower infection intensities following challenge and protected against anaemia in studies in laboratory animals.[13,15,17–19] On the basis of these studies, *Na*-GST-1 and *Na*-APR-1 were selected as lead vaccine candidates.

Both vaccines have been well tolerated and safe in clinical trials in which the antigens were administered separately to healthy adults. Two phase 1 trials of recombinant *Na*-GST-1 were done in the USA and Brazil, with the latter trial including participants living in both *N americanus*-endemic and non-endemic areas.[20]

Vaccinations in both trials were safe, well tolerated, and resulted in significant antigen-specific IgG responses.[20] Recombinant *Na*-APR-1 (M74) adjuvanted on Alhydrogel and administered with or without an aqueous formulation of the toll-like receptor-4 agonist, glucopyranosyl lipid A (GLA-AF), was tested in 40 healthy, hookworm-naive adults in a phase 1 trial in the USA and was found to be safe and well tolerated and to induce significant antigen-specific IgG responses.[21]

Based on preclinical studies,[15,17,18] it is expected that more than a single *N americanus* antigen will be required for an effective vaccine. Therefore, the clinical development plan for the human hookworm vaccine has been to incorporate at least two antigens into a final, co-formulated product. Co-administration of the two existing vaccine products should guide the choice of dose for each antigen.

The aims of this study were to assess the safety and immunogenicity of co-administered *Na*-GST-1 and *Na*-APR-1 (M74) vaccines in healthy Gabonese adults. To our knowledge, this trial is the first to present results for the hookworm vaccine *Na*-APR-1 (M74) adjuvanted with Alhydrogel from participants residing in an *N americanus*-endemic area.

Methods

Study design and participants

This randomised, controlled, double-blind, phase 1, dose-escalation trial was done at the Centre de Recherches Médicales de Lambaréné (Lambaréné, Gabon), in a region of Gabon where *N americanus* and other helminths are prevalent.[22,23] Healthy adults aged 18–50 years and living in Lambaréné or the surrounding areas were recruited. Fieldworkers approached potential candidates in their communities to explain the purpose of the study. Interested individuals were brought to the research centre for consenting procedures and assessment of eligibility. Eligible participants had to be in good general health, which was judged based on medical history, physical examination, and laboratory tests. Inclusion and exclusion criteria are listed in detail in the appendix (pp 70–71). All screening procedures were done within 90 days of enrolment. Clinically significant abnormalities, such as positive serological tests for HIV or hepatitis B surface antigen or abnormal haematological parameters, were reviewed with volunteers, and referral for follow-up care was provided.

Testing for intestinal helminths was done during screening by use of Kato-Katz fecal thick smear on stool samples. Individuals infected with soil-transmitted helminths (ie, hookworm, *Ascaris lumbricoides*, *Strongyloides stercoralis*, or *Trichuris trichiura*) were treated with 400 mg albendazole daily for 3 consecutive days. Individuals infected with *Schistosoma mansoni* or *Taenia* spp were treated with 60 mg/kg praziquantel. If other eligibility criteria were met (appendix pp 70–71), these individuals could be enrolled, with a minimum of 2 weeks between anthelmintic treatment and start of vaccinations. The purpose of treatment was to benefit participants, and to reduce possible effects of active infections on vaccination. Neither post-treatment retesting to confirm cure nor testing before second and third vaccinations were done. Written informed consent was obtained from all participants. In the case of impaired literacy, a literate impartial witness attested that informed consent was obtained. Before signing the informed consent document, a true or false questionnaire to evaluate consent comprehension was done. If the test was not passed after three attempts, the individual was not enrolled.

This study was approved by the National Ethics Committee of Gabon (#0033/2014/SG/CNE) and done under an investigational new drug application (IND#016184) to the US Food and Drug Administration. The study was done according to Good Clinical Practice guidelines. Reporting of the trial was done according to CONSORT criteria (appendix pp 68–69). The study protocol is provided in the appendix (pp 1–68).

Randomisation and masking

Participants were enrolled consecutively into two cohorts. In the first cohort, participants were randomly assigned to receive either 30 µg *Na*-GST-1 vaccine and 30 µg *Na*-APR-1 (M74) vaccine, both vaccines co-administered with Alhydrogel and GLA-AF, or Engerix B (GlaxoSmithKline Biologicals, Rixensart, Belgium) hepatitis B vaccine co-administered with sterile saline placebo (control group). In the second cohort, participants were randomly assigned to either receive 100 µg *Na*-GST-1 vaccine and 100 µg *Na*-APR-1 (M74) vaccine, both vaccines co-administered with Alhydrogel and GLA-AF, or to the control group.

Standard block randomisation was done, with each block of four containing one control; the randomisation list was provided to the study vaccine manager in a sealed envelope.

Hepatitis B vaccine was selected as the comparator because of its established safety record, similar physical appearance, and similar dosing schedule to the hookworm vaccines, and the benefit that it might provide to those randomly assigned to the control group. Saline was also administered to individuals who were randomly assigned to receive hepatitis B vaccine so that all participants would receive two injections (ie, one in each arm), thus maintaining blinding. Saline was chosen as it was decided that giving two different licensed vaccines (with the same vaccination schedule) would be logistically challenging and most mild injection-site reactions are unrelated to the antigen but instead due to the mechanical effects of bolus administration (eg, injection-site pain and small haematomas).

See Online for appendix

Vaccines were administered in a double-blind manner. Only the vaccine dispensers were aware of vaccine allocations. Furthermore, the content of syringes was concealed using opaque tape, and vaccinators were not involved in assessment of reactogenicity or adverse

events. Researchers who did antibody measurements were also masked to group assignment.

Procedures

Na-GST-1 vaccine was manufactured according to current good manufacturing practices and formulated on Alhydrogel (aluminium hydroxide adjuvant).[24,25] Since expression of refolded *Na*-APR-1 in *Escherichia coli* was hampered by issues of yield and protein aggregation,[18] recombinant *Na*-APR-1 was instead produced by infiltration of *Nicotiana benthamiana* tobacco plants with *Agrobacterium tumefaciens* strain GV3101, which was genetically engineered to express *Na*-APR-1 (M74).[26,27] To improve the stability of recombinant *Na*-APR-1 and to preclude digestion of host haemoglobin in vaccine recipients, two aspartic acid residues were mutated to alanines to make catalytically inactive *Na*-APR-1 (M74).[26]

Recombinant *Na*-GST-1 was manufactured as a 0·1 mg/mL suspension and adsorbed to 0·8 mg/mL Alhydrogel (Biosector; Frederikssund, Denmark) in a buffer of 10% glucose and 10 mM imidazole (pH 7·4) at Aeras Global Vaccine Foundation (Rockville, MD, USA).[24] Recombinant *Na*-APR-1 (M74) was supplied as a 0·1 mg/mL suspension adsorbed to 0·8 mg/mL of Alhydrogel in a solution containing 10 mM imidazole, 150 mM sodium chloride, and 0·3% Empigen BB (pH 7·4). *Na*-APR-1 (M74) expression was performed at the Fraunhofer Center for Molecular Biotechnology (Newark, DE, USA). Purification and vialling were done at the Walter Reed Army Institute of Research (Silver Spring, MD, USA).

GLA-AF was supplied as an aqueous solution in multidose vials containing 25 µg/mL of GLA without preservative. Both *Na*-GST-1/Alhydrogel and *Na*-APR-1 (M74)/Alhydrogel were mixed with 5 µg of GLA-AF within 24 h before vaccination for all dosage groups.

Engerix B hepatitis B vaccine was supplied in single-dose vials, with each 1·0 mL dose containing 20 µg of recombinant hepatitis B surface antigen and 500 µg of aluminium as aluminium hydroxide.

Vaccinations were administered via intramuscular injection in the deltoid muscle on study days 0, 28, and 180. On days of vaccination, each volunteer received two injections, one in each deltoid. Volunteers were followed up for 12 months after final vaccination.

Participant safety was monitored by an independent medical monitor and overseen by a safety monitoring committee that reviewed interim safety data before dose escalation to 100 µg. Study procedures are fully presented in the appendix (p 71).

IgG antibodies against *Na*-GST-1 and *Na*-APR-1 (M74) were measured in serum samples, collected at study days 0, 14, 28, 42, 56, 180, 194, 208, 270, and 360, by qualified indirect ELISA, which complied with applicable regulatory guidance.[28,29] Homologous standard reference sera were made by pooling sera collected from high IgG responders to the respective vaccine antigen from hookworm-naive Brazilian study participants[20] for *Na*-GST-1 and from hookworm-naive study participants in the USA for *Na*-APR-1 (M74).[21] Each standard reference serum was serially diluted in duplicate along 11 columns of each ELISA plate to generate dilution–response curves for IgG against *Na*-GST-1 or *Na*-APR-1 (M74) when using a mouse anti-human IgG. Dilution–response curves for IgG against each antigen were modelled into standard calibration curves using a four-parameter logistic log function as described previously.[15,16] The standard calibration curves were used to derive reactivity thresholds of IgG to each vaccine antigen (also referred to as limits of quantitation)[28] that were used to define IgG seroconversion to the vaccine antigens.

For qualified indirect ELISA, 96-well microtitre plates (Nunc Polysorp; ThermoFisher; Waltham, MA, USA) were incubated overnight at 4°C with either 100 µL of *Na*-GST-1 or *Na*-APR-1 (M74) diluted in coating buffer (phosphate-buffered saline, pH 7·2). Plates were then decanted and washed three times with phosphate-buffered saline and test serum samples from trial participants were added in duplicate at 1/1000 in dilution buffer containing phosphate-buffered saline-Tween 20 with 5% bovine serum albumin (Fitzgerald Industries International; North Action, MA, USA). Plates were sealed and incubated overnight at 4°C, after which they were removed, decanted, and washed five times with phosphate-buffered saline-Tween 20. Then, 100 µL of mouse anti-human IgG conjugated to horseradish peroxidase (Southern Biotech; Birmingham, AL, USA) was added as a secondary antibody at 1/1000 in dilution buffer. Plates were resealed and incubated at 4°C for 2 h, after which they were decanted and washed five times with phosphate-buffered saline-Tween 20. Plates were developed by adding 100 µL of o-phenylenediamine dihydrochloride (Sigma Aldrich; St Louis, MO, USA) for 30 min in the dark at room temperature and read at an optical density of 492 nm on a validated SpectraMax Plus 384 microplate reader (Molecular Devices; San Jose, CA, USA) with data collected using SOFTmax GXP PRO version 4 software (Molecular Devices). The mean of the optical density at 492 nm of each test sera duplicate was interpolated onto the standard calibration curve to derive the arbitrary units of anti-*Na*-GST-1 or anti-*Na*-APR-1 (M74) IgG.[30,31] Reactivity thresholds for anti-*Na*-GST-1 IgG or anti-*Na*-APR-1 (M74) IgG were obtained from the standard calibration cures, as discussed elsewhere.[30,31]

Outcomes

The primary objective of this study was to estimate the safety for each dose of co-administered *Na*-GST-1 and *Na*-APR-1 (M74). Secondary objectives were to determine the doses of *Na*-GST-1 and *Na*-APR-1 (M74) that generated the highest IgG antibody responses at day 194 and to assess antibody response duration.

Adverse event grading is presented in the appendix (p 71). Because GLA-AF was added to both experimental vaccines, the incidences of the following adverse events of special interest[32] were actively monitored throughout the study: neuroinflammatory disorders (eg, optic neuritis and multiple sclerosis), autoimmune disorders (eg, systemic lupus erythematosus and rheumatoid arthritis), gastrointestinal disorders (eg, inflammatory bowel disease), metabolic diseases (eg, autoimmune thyroiditis), vasculitides, and other autoimmune or inflammatory diseases. These adverse events of special interest, or potentially immune-mediated disorders, are not specific to GLA-AF but are a list of diagnoses monitored during trials of novel immunostimulants.[32]

Statistical analysis

This trial was not powered to detect statistically significant differences between groups. For safety analyses, an intention-to-treat population was used in which data from all enrolled participants were included, whereas for immunogenicity analyses, a per-protocol population was used, in which only data from participants who had received all scheduled vaccinations up to the analysis timepoint were included. The sample size of 32 was within the range commonly used in phase 1 trials for initial assessment of investigational vaccine safety and immunogenicity.

Safety data were reported as frequencies and percentages and compared by vaccine allocation (*Na*-GST-1 and *Na*-APR-1 [M74] *vs* control group) and dose cohort (30 µg *vs* 100 µg of antigen). These analyses were done using Stata 14.2. For clinical laboratory abnormalities, the mean and SD of the number of events per participant were calculated using Microsoft Excel for Mac 2018 (version 16.16.14).

To describe differences in antibody responses between groups, we calculated mean (with SDs) arbitrary units of IgG against each vaccine antigen by study day and dose and mean differences (with 95% CIs) between the experimental groups and control group. Control vaccine recipients for both dose cohorts were combined for the analyses. The number of seroresponders (with 95% CIs from Wilson score) for each antigen was calculated by summing those with IgG units above the reactivity threshold. Vaccine response distributions are presented as reverse cumulative distributions by study day (appendix pp 77–78). SAS version 9.4 was used for these analyses. Figures were plotted using GraphPad Prism version 7.04.

The trial is registered with ClinicalTrials.gov, NCT02126462.

Role of the funding source

The funder was engaged in study conduct according to EU standards, to ensure control over use of public funds in a transparent, accountable manner. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

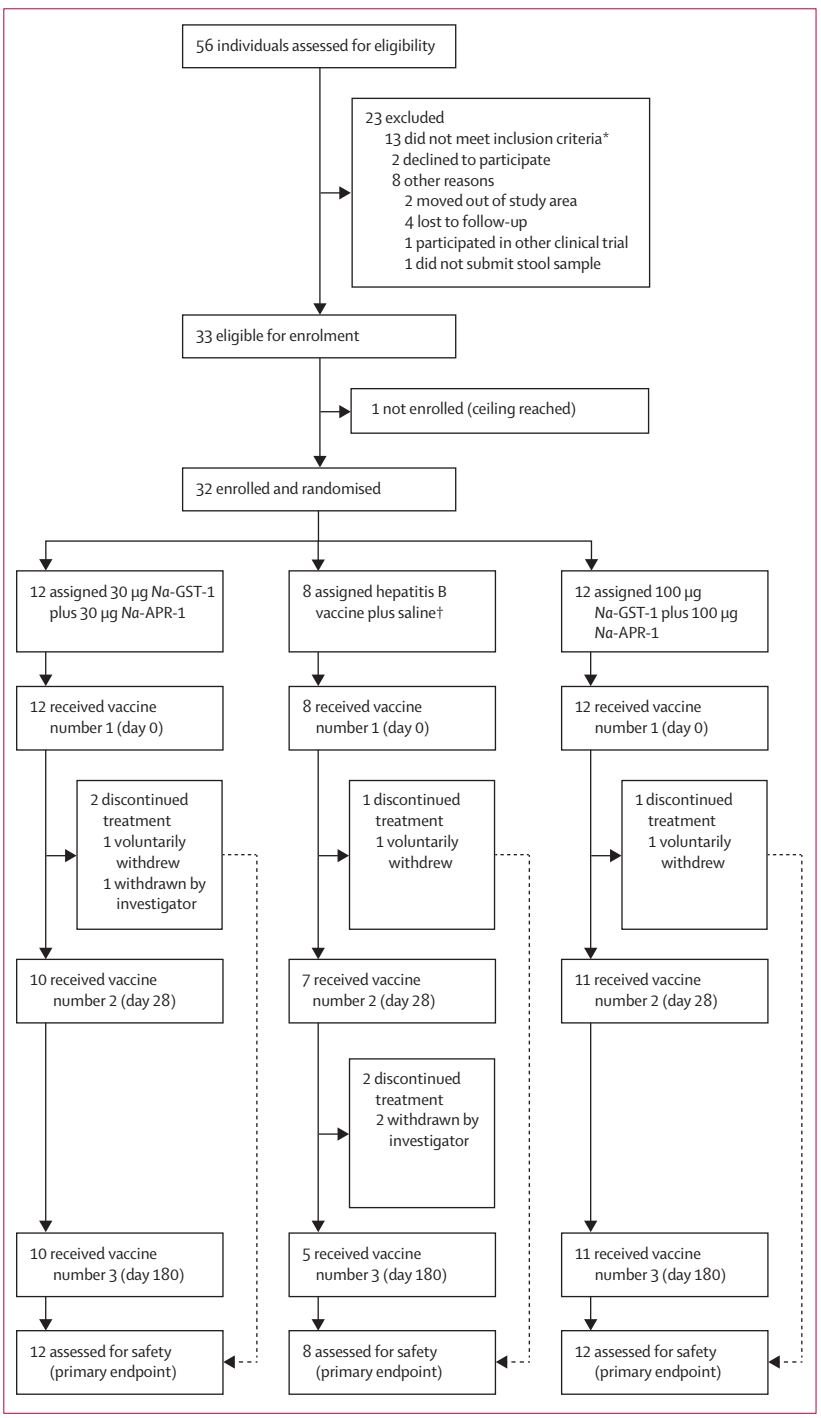

Figure 1: **Trial profile**
*Individuals had exclusionary medical or psychiatric conditions (one each with recurrent headache, recent blood transfusion, and recent weight loss) or abnormal laboratory test results (one positive for HIV, four positive for hepatitis B virus, two with abnormal complete blood count, and three with abnormal urinalysis).
†Participants in the control group received hepatitis B vaccine and are pooled across dose cohorts.

Results

Between Oct 27, 2014, and Jan 31, 2015, 56 volunteers were screened for eligibility, of whom 33 were eligible for enrolment and 32 were enrolled. One eligible volunteer was not enrolled as the enrolment ceiling of 32 participants had been reached (figure 1). 16 individuals each were enrolled into the 30 µg and 100 µg dose cohorts and randomly assigned to either receive the experimental vaccines (24 individuals) or to the control group (eight individuals; figure 1).

24 (75%) men and eight (25%) women were enrolled, with a median age of 22 years (range 18–50; IQR 20–28; table 1). Four participants were positive for hookworm eggs at screening, two in the 30 µg *Na*-GST-1 and *Na*-APR-1 group and two in the 100 µg *Na*-GST-1 and *Na*-APR-1 group (one of whom was lost to follow-up after the first vaccination).

Six participants did not receive all three planned vaccinations (figure 1). Of these, three (50%) were lost to follow-up or withdrew consent for personal reasons, and the remaining three were withdrawn from receiving additional vaccinations by the investigator for medical reasons (two participants because they received tetanus IgG during the vaccination phase of the trial and one, who was randomly assigned to the control group, because of pregnancy).

Both study vaccines were well tolerated in both dosage groups. No vaccine-related serious adverse events or adverse events of special interest were recorded. No

	Control group (n=8)	30 µg *Na*-GST-1 and *Na*-APR-1 (n=12)	100 µg *Na*-GST-1 and *Na*-APR-1 (n=12)
Age, years			
Median (IQR)	22 (21–23)	28 (21–36)	22 (20–23)
Mean (SD)	24·3 (7·7)	29·3 (10·5)	23·2 (6·8)
Range	19–43	18–50	19–44
Sex			
Male	6 (75%)	9 (75%)	9 (75%)
Female	2 (25%)	3 (25%)	3 (25%)
Hookworm egg positive*	0	2 (17%)	2 (17%)

Data are n (%) unless otherwise indicated. *In the 30 µg group, one individual tested positive for hookworm and one for hookworm and *Strongyloides stercoralis*; in the 100 µg group, one individual tested positive for hookworm (but was lost to follow-up) and one for hookworm, *Trichuris trichuria*, and *Taenia* spp.

Table 1: **Baseline characteristics of the intention-to-treat population**

	Control group (n=8)*			30 µg *Na*-GST-1 and *Na*-APR-1 (n=12)			100 µg *Na*-GST-1 and *Na*-APR-1 (n=12)		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Injection site									
Pain	5 (63%)	5 (63%)	0	11 (92%)	9 (75%)	0	11 (92%)	7 (58%)	0
Erythema	0	0	0	0	0	0	1 (8%)	0	0
Swelling	0	1 (13%)	0	2 (17%)	0	0	2 (17%)	0	0
Systemic									
Arthralgia	2 (25%)	1 (13%)	0	0	0	0	1 (8%)	0	0
Fever	3 (38%)	0	1 (13%)	0	1 (8%)	1 (8%)	0	0	0
Headache	6 (75%)	3 (38%)	0	5 (42%)	4 (33%)	0	8 (67%)	1 (8%)	0
Myalgia	2 (25%)	2 (25%)	0	2 (17%)	2 (17%)	0	1 (8%)	0	0
Nausea	3 (38%)	0	0	4 (33%)	0	0	3 (25%)	0	0
Vomiting	0	0	0	1 (8%)	0	0	0	0	0
Clinical laboratory parameters									
Decreased haemoglobin	3	1	3	8	4	0	5	2	0
Decreased neutrophil count	3	0	1	6	2	0	5	0	0
Decreased platelet count	3	0	2	3	1	2	2	0	3
Decreased white blood cell count	1	0	0	3	0	0	0	0	0
Increased white blood cell count	2	0	0	2	0	0	1	0	0
Increased alanine aminotransferase	1	0	0	1	1	0	1	0	0
Increased creatinine	0	0	0	0	0	0	0	0	0

For injection site and systemic adverse events, data are n (%). Data show the number of participants with at least one event reported in this severity classification with this adverse event between the time of starting vaccination up to 14 days post-vaccination. For clinical laboratory parameters, data are the number of events reported.
*The control group received hepatitis B vaccine and saline.

Table 2: **Frequency of solicited adverse events and clinical laboratory adverse events by dose for all vaccinations in the intention-to-treat population**

participants were withdrawn or had vaccinations suspended because of vaccine-related adverse events. Mild-to-moderate injection-site pain was common across cohorts, but less frequent in the control group (table 2). The most frequent solicited systemic events were mild-to-moderate headache, nausea, and myalgia (table 2). One participant had mild injection-site erythema after the second vaccination with 100 µg *Na*-APR-1 (M74). We observed no differences in the frequencies of adverse events between the hookworm and comparator vaccine groups, although because of the small group numbers no formal statistical comparisons were done.

Two severe episodes of fever occurred (one each in the 30 µg *Na*-GST-1 and *Na*-APR-1 and comparator vaccine groups); both episodes were considered unlikely to be related to vaccination because of concomitant diagnoses

	30 µg		100 µg		Control group*	
	Na-GST-1	*Na*-APR-1 (M74)	*Na*-GST-1	*Na*-APR-1 (M74)	*Na*-GST-1	*Na*-APR-1 (M74)
Day 0 (first dose)						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	2·53 (0)	7·49 (5·36)	3·58 (2·67)	22·08 (37·65)	2·53 (0)	13·43 (15·20)
Mean difference *vs* control group (95% CI)	0 (–2·00 to 2·00)	–5·93 (–35·21 to 23·34)	1·05 (–0·91 to 3·02)	8·65 (–20·18 to 37·48)	..	..
Day 14						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	2·80 (0·86)	7·39 (5·53)	5·48 (4·44)	61·40 (164·79)	3·09 (1·27)	9·79 (10·16)
Mean difference *vs* control group (95% CI)	–0·29 (–3·72 to 3·13)	–2·4 (–125·67 to 120·87)	2·39 (–0·99 to 5·76)	51·61 (–69·78 to 173·00)	..	..
Day 28 (second dose)						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	2·82 (0·94)	7·52 (5·20)	4·33 (3·23)	25·77 (47·10)	2·53 (0)	9·14 (7·73)
Mean difference *vs* control group (95% CI)	0·30 (–2·21 to 2·80)	–1·61 (–37·18 to 33·96)	1·81 (–0·66 to 4·27)	16·63 (–18·4 to 51·66)	..	..
Day 42						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	14·20 (9·52)	8·41 (5·95)	43·41 (53·83)	29·88 (35·50)	2·53 (0)	10·45 (9·29)
Mean difference *vs* control group (95% CI)	11·68 (–29·10 to 52·46)	–2·05 (–29·26 to 25·17)	40·88 (0·72 to 81·04)	19·43 (–7·37 to 46·23)	..	..
Day 56						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	11·44 (9·05)	8·54 (5·85)	33·48 (48·14)	39·63 (65·60)	2·53 (0)	7·67 (6·26)
Mean difference *vs* control group (95% CI)	8·91 (–27·62 to 45·44)	0·87 (–48·41 to 50·15)	30·95 (–5·02 to 66·92)	31·96 (–16·56 to 80·49)	..	..
Day 180 (third dose)						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	3·55 (1·69)	6·87 (5·07)	6·65 (6·04)	18·03 (31·32)	2·53 (0)	8·47 (3·75)
Mean difference *vs* control group (95% CI)	1·03 (–3·64 to 5·69)	–1·60 (–25·34 to 22·14)	4·13 (–0·47 to 8·72)	9·56 (–13·82 to 32·93)	..	..
Day 194						
Sample size, n	10	10	11	11	5	5
Mean arbitrary units of IgG (SD)	320·32 (422·42)	18·15 (14·66)	382·36 (311·25)	169·82 (195·75)	2·53 (0)	6·53 (2·99)
Mean difference *vs* control group (95% CI)	317·80 (–61·30 to 696·90)	11·62 (–135·00 to 158·24)	379·80 (6·50–753·10)	163·29 (18·91 to 307·67)	..	..
Day 208						
Sample size, n	10	10	10	10	5	5
Mean arbitrary units of IgG (SD)	199·77 (311·44)	13·02 (8·10)	331·69 (366·39)	119·84 (143·41)	2·53 (0)	6·31 (3·63)
Mean difference *vs* control group (95% CI)	197·20 (–152·10 to 546·60)	6·70 (–97·67 to 111·07)	329·20 (–20·20 to 678·50)	113·53 (9·16 to 217·90)	..	..
Day 270						
Sample size, n	10	10	11	11	4	4
Mean arbitrary units of IgG (SD)	68·71 (87·69)	11·41 (9·81)	95·78 (98·1)	53·47 (76·33)	2·53 (0)	7·50 (4·51)
Mean difference *vs* control group (95% CI)	66·19 (–40·21 to 172·58)	3·91 (–59·73 to 67·56)	93·25 (–11·75 to 198·26)	45·97 (–16·84 to 108·78)	..	..
Day 360						
Sample size, n	10	10	9	9	5	5
Mean arbitrary units of IgG (SD)	22·50 (22·93)	9·99 (7·73)	32·24 (30·70)	30·74 (47·22)	2·53 (0)	19·46 (26·65)
Mean difference *vs* control group (95% CI)	19·98 (–7·55 to 47·51)	–9·47 (–45·68 to 26·73)	29·72 (1·68 to 57·76)	11·28 (–25·59 to 48·15)	..	..

n indicates the number of participants vaccinated with all three doses of *Na*-GST-1 or *Na*-APR-1 (M74) and with an assessment on the study day. *Participants in the control group received hepatitis B vaccine and are pooled across dose cohorts.

Table 3: **Comparison of IgG antibody levels among the three groups for anti-*Na*-GST-1 and anti-*Na*-APR-1 (M74)**

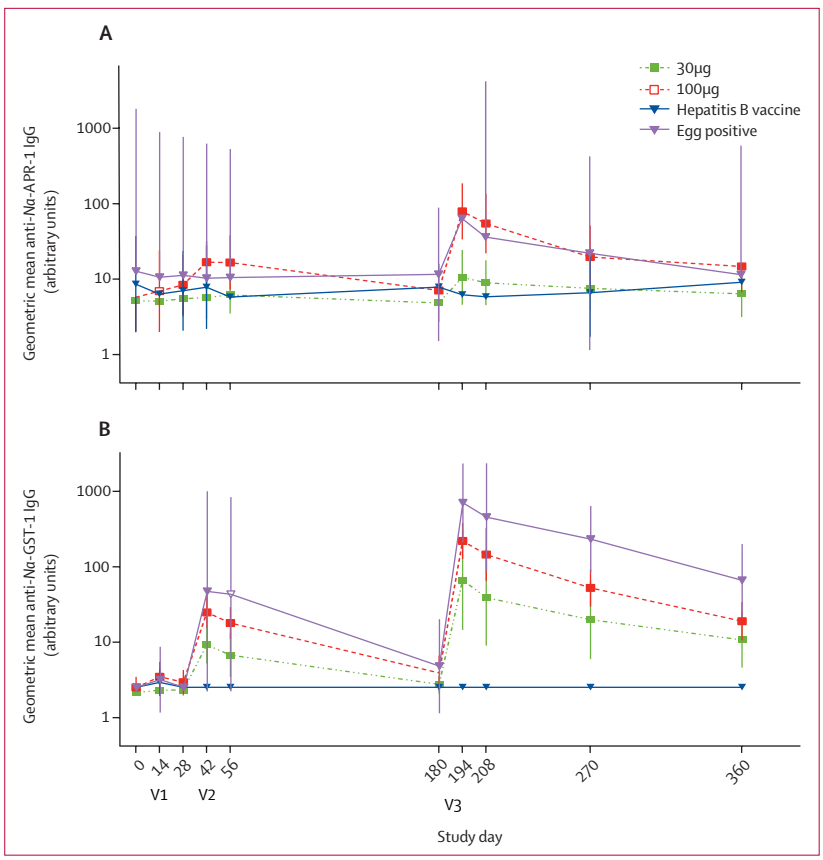

***Figure 2*: Mean levels of IgG against *Na*-APR-1 (M74; A) and *Na*-GST-1 (B) in study participants immunised with *Na*-GST-1 and *Na*-APR-1 (M74), as measured by ELISA**

Data were stratified by the comparator group, dose group (without participants who tested positive for hookworm eggs at screening), and egg-positive group. Error bars represent 95% CIs. V1=first vaccination. V2=second vaccination. V3=third vaccination.

of *Plasmodium falciparum* malaria. We observed no other severe solicited events.

We observed no serious adverse events related to haematology parameters (ie, increased alanine aminotransferase) or clinical chemistry laboratory abnormalities (ie, increased creatinine; table 2). Most laboratory adverse events were asymptomatic, transient, and resolved spontaneously. The most commonly observed clinical laboratory adverse events were decreases in haemoglobin concentration from baseline (day 0), with 26 events in total: 12 in the 30 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 1·0 event per participant [SD 1·15]), seven in the 100 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 0·59 events per participant [0·64]), and seven in the control group (mean of 0·88 events per participant [0·78]). All decreases were mild or moderate in severity, except for three participants in the control group who had a severe decrease (2·1–5·0 g/dL) in haemoglobin concentration, which was associated with concurrent diagnoses of *P falciparum* malaria.

17 decreases in absolute neutrophil count were reported (table 2), eight in the 30 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 0·67 events per participant [SD 0·85]), five in the 100 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 0·42 events per participant [0·64]), and four in the control group (mean of 0·50 events per participant [1·0]). Most of these decreases were mild in severity, although one severe decrease (500–750 per µL) was observed in one participant in the control group and two moderate decreases (750–1000 per µL) were observed in two participants in the 30 µg *Na*-GST-1 and *Na*-APR-1 group.

16 decreases in platelet count were reported during the study, including six in the 30 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 0·33 events per participant [SD 0·47]), five in the 100 µg *Na*-GST-1 and *Na*-APR-1 group (mean of 0·42 events per participant [0·64]), and five in the control group (mean of 0·63 events per participant [0·69]; table 2). Severe decreases in platelet count were seen in all three groups, although none of these were considered related to vaccination; most were attributed to concomitant malaria diagnoses.

52 unsolicited adverse events possibly, probably, or definitely related to the study vaccine occurred during the study (appendix p 72). No differences were observed in the frequencies of unsolicited vaccine-related adverse events between vaccine groups; however, because of small sample sizes, no formal statistical comparisons were done. One severe unsolicited vaccine-related adverse event was reported, which was fever in a participant who received the Engerix B hepatitis B vaccine. No participant who received the hookworm vaccines had a severe, unsolicited, vaccine-related adverse event.

For both *Na*-APR-1 (M74) and *Na*-GST-1, peak IgG levels were observed at day 194, 2 weeks after the third vaccination (table 3, figure 2). At this timepoint, higher mean levels of IgG against *Na*-APR-1 (M74) were observed in participants who received the 100 µg dose (169·82 arbitrary units) than in those who received the 30 µg dose (18·15 arbitrary units; table 3, figure 2A). Similarly, higher anti-*Na*-GST-1 IgG was seen at peak response in participants who received 100 µg *Na*-GST-1 (382·36 arbitrary units) than in those who received the 30 µg dose (320·32 arbitrary units; table 3; figure 2B).

14 (54%) of 26 participants were seropositive for anti-*Na*-APR-1 (M74) IgG on day 0 (before vaccine administration), meaning they had IgG levels above the reactivity threshold of 4·89 arbitrary units. One (4%) evaluable participant was seropositive for anti-*Na*-GST-1 IgG on day 0, with IgG levels above the reactivity threshold of 6·77 arbitrary units.

For ten participants who received all three doses of 30 µg *Na*-APR-1, no change from baseline in IgG was observed 14 days after the first vaccination, whereas increases from the day of vaccination were seen 2 weeks after the second and third vaccine doses, with the greatest increase seen from the third dose (mean IgG arbitrary units 6·87 [SD 5·07]) to study day 194 (18·15 [14·66]; table 3, figure 2A; appendix p 75). By study day 360, mean

arbitrary units of IgG had decreased to almost baseline levels. Eight (80%) participants receiving 30 µg *Na*-APR-1 (M74) were seropositive at study day 194 and seven (70%) were seropositive at study day 360 (table 4).

Five (45%) of 11 participants who received all three doses of 100 µg *Na*-APR-1 (M74) had anti-*Na*-APR-1 (M74) IgG levels above the reactivity threshold at study day 0 (table 4; appendix p 77). A change from baseline in anti-*Na*-APR-1 (M74) IgG was observed 2 weeks after the first vaccine dose, with a smaller increase seen after the second dose (table 3, figure 2A). The mean IgG level decreased to 18·03 arbitrary units (SD 31·32) by study day 180. After the third vaccine dose, the mean IgG against *Na*-APR-1 (M74) increased by 151·8 arbitrary units to study day 194 (169·82 arbitrary units [SD 195·75]). IgG decreased to a mean of 30·74 arbitrary units (SD 47·22) by study day 360, or 10·2 arbitrary units higher than baseline (appendix p 76). All 11 participants receiving 100 µg *Na*-APR-1 (M74) were seropositive 14 days after administration of the third vaccine dose, which was maintained in nine evaluable participants at 6 months after vaccination (table 4).

For ten participants vaccinated with 30 µg *Na*-GST-1, we observed no change from baseline in mean anti-*Na*-GST-1 IgG after the first vaccine dose (table 3, figure 2B). After the second vaccine dose, the mean IgG level increased from 2·82 arbitrary units (SD 0·94) on day 28 to 14·2 arbitrary units (9·52) on day 42, with seven participants (70%) becoming seropositive (tables 2, 3). From the third vaccine dose, mean anti-*Na*-GST-1 IgG levels increased by 316·7 arbitrary units to study day 194, but then decreased to 22·5 arbitrary units (SD 22·93) on day 360. Eight (80%) participants were seropositive 14 days after the third vaccine dose and seven (70%) were seropositive 6 months after. One participant showed no increase in IgG against *Na*-GST-1 after vaccination with 30 µg *Na*-GST-1.

For 11 participants who received three doses of 100 µg *Na*-GST-1, a small change from baseline was observed in mean anti-*Na*-GST-1 IgG after the first vaccine dose (table 3, figure 2B). A bigger increase was observed after the second vaccine dose, although the mean IgG level had decreased again by day 180 (table 3, figure 2B; appendix p 77). All 11 evaluable participants were seropositive between the second and third set of vaccinations (table 4). After the third vaccine dose, the mean IgG level increased by 375·7 arbitrary units (from 6·65 arbitrary units [SD 6·04] on day 180 to 382·36 arbitrary units [311·25] on day 194), decreasing again to 32·24 arbitrary units (SD 30·7) on day 360 (table 3, figure 2B). All 11 participants were seropositive at day 194, which was maintained in all nine evaluable participants at day 360 (table 4).

When pooled, all participants among the two vaccine groups who were positive for hookworm eggs at screening showed similar *Na*-APR-1 IgG responses to those in the 100 µg group overall (figure 2). By contrast, all participants in the 30 µg and 100 µg vaccine groups who were egg positive at screening had higher anti-*Na*-GST-1 IgG levels after the second and third vaccine doses than did those who were egg negative (figure 2).

	30 µg		100 µg		Control group*	
	N†	n (%)‡§	N†	n (%)‡§	N†	n (%)‡§
***Na*-APR-1 (M74)**						
0	10	6 (60%, 31–83)	11	5 (45%, 21–72)	5	3 (60%, 23–88)
14	10	6 (60%, 31–83)	11	5 (45%, 21–72)	5	3 (60%, 23–88)
28	10	7 (70%, 40–89)	11	8 (73%, 43–90)	5	3 (60%, 23–88)
42	10	6 (60%, 31–83)	11	10 (91%, 62–98)	5	3 (60%, 23–88)
56	10	8 (80%, 49–94)	11	10 (91%, 62–98)	5	3 (60%, 23–88)
180	10	6 (60%, 31–83)	11	8 (73%, 43–90)	5	4 (80%, 38–96)
194	10	8 (80%, 49–94)	11	11 (100%, 74–100)	5	4 (80%, 38–96)
208	10	8 (80%, 49–94)	10	10 (100%, 72–100)	5	3 (60%, 23–88)
270	10	8 (80%, 49–94)	11	10 (91%, 62–98)	4	3 (75%, 30–95)
360	10	7 (70%, 40–89)	9	9 (100%, 70–100)	5	3 (60%, 23–88)
***Na*-GST-1**						
0	10	0 (0%, 0–28)	11	1 (9%, 2–38)	5	0 (0%, 0–43)
14	10	0 (0%, 0–28)	11	2 (18%, 5–48)	5	0 (0%, 0–43)
28	10	0 (0%, 0–28)	11	1 (9%, 2–38)	5	0 (0%, 0–43)
42	10	7 (70%, 40–89)	11	11 (100%, 74–100)	5	0 (0%, 0–43)
56	10	6 (60%, 31–83)	11	11 (100%, 74–100)	5	0 (0%, 0–43)
180	10	1 (10%, 2–40)	11	6 (55%, 21–72)	5	0 (0%, 0–43)
194	10	8 (80%, 49–94)	11	11 (100%, 74–100)	5	0 (0%, 0–43)
208	10	8 (80%, 49–94)	10	10 (100%, 72–100)	5	0 (0%, 0–43)
270	10	8 (80%, 49–94)	11	11 (100%, 74–100)	4	0 (0%, 0–49)
360	10	7 (70%, 40–89)	9	9 (100%, 70–100)	5	0 (0%, 0–43)

*Participants in the control group received hepatitis B vaccine and are pooled across cohorts. †Number of participants in the immunogenicity per-protocol population at the study visit analysed. ‡Number of participants with seroresponse (%, 95% CI); seroresponse was defined as arbitrary units of ELISA IgG above 4·89 for *Na*-APR-1 (M74) and 6·77 for *Na*-GST-1. §95% CI from Wilson score.

Table 4: **Proportion of seropositive participants by study day for *Na*-APR-1 (M74) and *Na*-GST-1**

Discussion

To our knowledge, our study describes the first phase 1 clinical trial of two novel vaccine candidates against human hookworm infection (*Na*-APR-1 [M74] and *Na*-GST-1) done in sub-Saharan Africa. The study also describes, for the first time, the safety and immunogenicity of *Na*-APR-1 (M74) administered to healthy adult residents of an *N americanus*-endemic area. Co-administration of these candidate hookworm vaccines was safe and well tolerated, and antigen-specific IgG antibodies were induced to both vaccine antigens. Antibody levels peaked 2 weeks after the third vaccination and subsequently declined until the end of the study, although they remained significantly higher than baseline at day 360 for both antigens.

Na-GST-1 and *Na*-APR-1 (M74) are second-generation recombinant hookworm vaccine antigens. Their presumptive mechanism of action is to induce antibodies that are ingested by adult hookworms to block the

digestive enzymatic activities of each antigen.[15,16,18] These enzymes in the hookworm intestinal tract were targeted because of the results observed for the first-generation hookworm vaccine, *N americanus Ancylostoma*-secreted protein-2 (*Na*-ASP-2), a recombinant protein version of an infective larval excretory or secretory product released after larval skin penetration. Although this vaccine was safe and immunogenic in an area non-endemic for *N americanus* (the USA),[33] it induced generalised urticaria in a subsequent trial in an endemic area of Brazil, a reaction associated with pre-existing IgE against *Na*-ASP-2, probably from previous *N americanus* infections.[30] A subsequent serosurvey of residents in endemic areas of Brazil showed that IgE against *Na*-ASP-2 was common in both adults and children.[30] Development of *Na*-ASP-2 was halted, and the second generation of hookworm vaccines was developed. As a precaution, before studies of these second-generation hookworm vaccines in *N americanus*-endemic areas, sera from residents of endemic areas in Brazil were tested against *Na*-APR-1 (M74) and *Na*-GST-1. No detectable levels of IgE to *Na*-GST-1 and only minimal levels of IgE to *Na*-APR-1 were found.[18] In the current study, no vaccine-related allergic reactions were observed.

Several participants in this study in Gabon had detectable pre-vaccination IgG to *Na*-APR-1, *Na*-GST-1, or both antigens, whereas pre-vaccination IgG against *Na*-GST-1 was not observed in the Brazilian phase 1 trial of this vaccine.[20] However, we found the presence of pre-existing IgG to *Na*-APR-1 (M74) or *Na*-GST-1 had no effect on IgG responses after vaccination. Positivity for hookworm eggs at screening had no effect on post-vaccination IgG levels for *Na*-APR-1 (M74), but antibody levels for *Na*-GST-1 appeared to be higher in participants with hookworm eggs at screening than in those without. Nevertheless, the 95% CIs for IgG levels for these subgroups overlapped considerably, probably due to the small number of egg-positive participants; therefore, no definitive conclusions can be drawn from these data. The patterns of IgG response to *Na*-APR-1 (M74) and *Na*-GST-1 observed in Gabonese participants were similar to those observed in participants vaccinated with only *Na*-APR-1 (M74) in the USA and participants vaccinated with only *Na*-GST-1 in both the USA and Brazil.[33] In all studies, IgG to each vaccine antigen peaked 2 weeks after the third vaccine dose.

In the present trial, *Na*-APR-1 (M74) and *Na*-GST-1 were co-administered with GLA-AF containing synthetic monophosphoryl lipid A, a toll-like receptor-4 agonist.[34] In clinical trials in healthy Brazilian and American adults, addition of GLA-AF to the Alhydrogel formulation of *Na*-GST-1 did not result in an increase in IgG responses compared with the Alhydrogel formulation administered alone.[20] In our study, the effect of GLA-AF on IgG responses could not be determined, as all hookworm vaccine doses were administered with this immunostimulant.

Regarding comparisons between IgG responses to *Na*-GST-1 and *Na*-APR-1, the units used to quantify these responses cannot be directly compared between the antigens, since arbitrary units are not equivalent to the mass value of antibodies in a sample. Although arbitrary units of sera from the same individual at different timepoints or different individuals at the same timepoint can be compared for a given antigen, they should not be directly compared between antigens.

A limitation of this study is possible false-negative screening results for soil-transmitted helminths and *Schistosoma* spp. Screening for intestinal helminths was done by Kato-Katz faecal thick smear, the primary diagnostic tool recommended by WHO for soil-transmitted helminth and trematode (*S mansoni*) infections. Participants found to be egg negative at screening were enrolled without pre-treatment with albendazole or praziquantel. A distortion of pre-vaccination and post-vaccination antibody levels could have resulted from hypothetical false negatives, although the extent to which such a distortion might have occurred is indeterminable.

In conclusion, *Na*-GST-1 and *Na*-APR-1 (M74), given with Alhydrogel and GLA-AF, were safe, well tolerated, and immunogenic when co-administered to adults living in *N americanus*-endemic areas of Gabon. IgG antibodies were produced to both vaccine antigens, with the peak of the antibody response 2 weeks after the third vaccine dose. A study of both vaccine candidates in healthy children in Gabon is underway (NCT02839161), as well as a phase 2 vaccination-challenge study in the USA using a controlled human hookworm infection model (NCT03172975).

Contributors

DJD, MPG, AAA, JMB, MEB, PJH, and RvL conceived the study. GL, JMB, and MM did the formal analysis. RvL, PJH, JMB, MEB, DJD, MPG, and PGK acquired the funding. AAA, SGdV, FJZ, YJH, J-CDA, ABH, and EBB did the clinical investigation. MML, JMB, and GL did the laboratory investigation. DJD, GL, JMB, PJH, MEB, and MPG devised the methodology. AAA, DJD, MPG, and PGK supervised the study. JMB, GL, and SGdV prepared the figures. SGdV wrote the original draft of the manuscript. SGdV, MPG, DJD, GL, JMB, AAA, MEB, and PJH reviewed and edited the manuscript. All authors contributed to the final version of the manuscript and approved it for publication.

HookVac Consortium collaborators

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Declaration of interests

PJH, MEB, DJD, and JMB have a patent pending on a multivalent anthelmintic vaccine (US8211438B2). All other authors declare no competing interests.

Data sharing

Deidentified individual participant data that underlie the results reported in this Article will be made available immediately after publication. The study protocol, informed consent form, and clinical study report will also be made available at this time.

For access to the **individual participant data that underlie the results reported in this Article** see https://clinicaltrials.gov/ct2/show/NCT02126462?term=Na-GST-1&draw=2&rank=4.

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